3-17-93

Guideline Series 83-3: Developmental Toxicity

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DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity; Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

Tox Chem. No.: 039

PC Code: 005101

CAS No.: 1918-02-1

MRID No.: 424609-01

TEST MATERIAL: 4-Amino-3,5,6 trichloropicolinate triisopropanolamine salt

SYNONYMS: Picloram TIPA; Amdon®; Borolin®; K-Pin®

SPONSOR: DowElanco, Indianapolis, IN

STUDY NUMBER: K-049877-015

TESTING FACILITY: Dow Chemical Company, Midland, MI

TITLE OF REPORT: Picloram Triisopropanolamine Salt: Oral Gavage Teratology Study in New Zealand White Rabbits.

AUTHORS: U. Vedula, K.E. Stebbins, and W.J. Breslin

REPORT ISSUED: August 11, 1992

CONCLUSIONS: A developmental toxicity study was conducted in which two groups of New Zealand White Rabbits were administered picloram TIPA via gavage on gestational days (GDs) 7-19, inclusively. In the first group (Phase I), animals received doses of 0, 180, 538, or 1,000 mg/kg/day (corresponding to 0, 100, 300, and 556 mg/kg/day of picloram acid). In the second group (Phase II), animals received doses of 0, 54, 180, 538, or 1,000 mg/kg/day (corresponding to 0, 30, 100, 300, and 558 mg/kg/day of picloram acid). Maternal toxicity was observed in both studies at ≥180 mg/kg/day and was manifested as an increased rate of abortions (1,000 mg/kg/day); increased incidences of clinical signs (538 and 1,000 mg/kg/day); and decreased food consumption and body weight gain (180, 538, and 1,000 mg/kg/day). Based on these results, the NOEL and LOEL for maternal toxicity were 54 and 180 mg/kg/day, respectively.

Developmental toxicity was not observed at any dose level in the Phase I or Phase II study. Consequently, the NOEL for developmental toxicity was 1,000 mg/kg/day (limit dose).

CLASSIFICATION: Core Guideline Data. This study meets the guideline requirements set forth under EPA Guideline Series 83-3 for a developmental toxicity study in rabbits.

A. MATERIALS

Test Compound

Purity:	61.02% (34.05% by weight as the acid equivalent)
Description:	Aqueous solution
Lot number:	AGR 276453
Receipt date:	Not reported
Contaminants:	Not reported

Vehicle: Distilled/deionized water

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Hazleton Research Products, Inc., Kalamazoo, MI
Age:	6-8 months on GD 0
Weight:	3250-4250 g on GD 0
Males used:	Not reported

B. STUDY DESIGN

This study was designed to assess the potential of picloram TIPA to cause developmental toxicity when administered daily via gavage from GDs 7-19, inclusively.

Insemination: Following at least 2 weeks of acclimation, females were artificially inseminated with the day of insemination considered GD 0. Following insemination, the does were given an intravenous injection of 100 I.U. Human Chorionic Gonadotropin (source not reported).

Animal Husbandry: Animals were fed diet of 8 oz/day (Purina® Certified Laboratory Rabbit Chow No. 5322). Tap water was available ad libitum throughout the study. Animals were maintained in an environmentally controlled room. Temperature and humidity were not reported.

Group Arrangement: Animals were assigned to dose groups using a computer-generated randomization procedure as follows:

Test Group	Phase I Dose Level (mg/kg/day)	Phase II Dose Level (mg/kg/day)	Number Animals Assigned Per Group
Control	0	0	18
Low-dose	180	54	18
Low Mid-dose	--	180	18
High Mid-dose	538	538	18
High-dose	1,000	1 000	18

Dose Administered: Doses were administered daily via gavage from GD 7 through GD 19 in a volume of 2 ml/kg. Individual doses were calculated based on the most recently recorded body weight data. Dosing solutions were prepared once prior to the start of the study. Analyses for concentration and homogeneity of the dosing solutions were performed prior to and after the dosing period. Stability had been determined previously.

Dose Rationale: Concentrations of the doses were selected based upon a range-finding teratology study (Vedulla et al., 1991) in which does were administered the test compound at 0, 180, 538, or 896 mg/kg/day from GD 7-19, inclusively. Neither maternal nor developmental toxicity was observed.

Observations: Animals were observed daily for mortality, moribundity and clinical signs. Body weight data were recorded on GDs 0, 7-19, 20, and 28. Food consumption data were recorded daily throughout the test period during Phase II of the study. On GD 28 does were sacrificed by an intravenous injection of Beuthanasia-D Special® (Scherring Corporation) and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- A limited gross pathology examination
- Gravid uterine weight
- Number of corpora lutea
- Number of implantation sites
- Numbers of resorptions and live and dead fetuses
- Liver (with gallbladder) and kidney weights

The uteri from apparently nonpregnant does were stained with 10% sodium sulfide solution to detect early embryonic loss.

Examination of all live fetuses included the following:

- Individual fetal weight and sex
- External anomalies

- Visceral anomalies (including fresh examination of the brain) using the method described by Staples (1974)

- Skeletal anomalies using the method described by Dawson (1926)

Statistical Analysis: The following methods were used.

- Maternal body weight and body weight gain, organ weights, and fetal body weight--Bartlett's test, ANOVA, and Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction

- Pre-implantation loss, resorptions, and fetal anomalies--Wilcoxon test with Bonferroni's correction

- Numbers of corpora lutea and implantation sites and litter size--ANOVA and Wilcoxon Rank-Sum test with Bonferroni's correction

- Pregnancy rates--Fisher's exact test

- Fetal sex ratios--Binomial distribution test

- Food consumption (Phase II)--Descriptive statistics only

Compliance

- A signed Statement of Data Confidentiality Claims, dated August 5, 1992, was provided.

- A signed Statement of Compliance with EPA, OECD, and MAFF GLPs, dated August 5 and 11, 1992, was provided.

- A signed Quality Assurance Statement, dated August 3, 1992, was provided.

C. RESULTS

Test Material Analysis

Analyses conducted for concentration of pre-study solutions in Phase I were 112%, 114%, and 115% of target at 180, 538, and 1,000 mg/kg/day, respectively. Post-study concentrations were 97%, 97%, and 99%, respectively at the same dose levels. Homogeneity analyses were 112% and 115% of target at the low- and high-dose, respectively.

Phase II prestudy analyses for concentration were 98%, 100%, 100%, and 98% of target at 54, 180, 538, and 1,000 mg/kg/day, respectively. Post-study concentrations were 140%, 124%, and 113% at 54, 538, and 1,000 mg/kg/day, respectively. Homogeneity analyses were 100% and 98% of target at the low- and high-dose levels, respectively.

The test compound had previously been shown to be stable in the vehicle for a period of at least 45 days.

Maternal Toxicity

Mortality: No mortality was observed in either Phase I or Phase II studies.

Abortion: Compound-related abortions occurred at 1,000 mg/kg/day. In Phase I, one doe aborted on GD 22 following severe weight loss during the dosing period. Necropsy revealed a pale liver, decreased ingesta, and blood in the urine. In Phase II, two does aborted on GDs 22 and 23 and one doe delivered early (just prior to necropsy). Necropsies did not reveal causes for abortion.

Clinical Observations: Compound-related effects were observed at 538 and 1,000 mg/kg/day. A summary of clinical signs during Phase II is presented in Table 1.

In Phase I (Table 1), 1, 1, 6, and 5 does showed decreased feces at 0, 180, 538, and 1,000 mg/kg/day, respectively. In Phase II, 2, 4, 5, 13, and 18 does showed decreased feces at 0, 54, 180, 538, and 1,000 mg/kg/day, respectively. In addition at the highest dose level the following clinical signs increased: soft feces, mucoid feces, perineal staining, and blood in pan. Incidental signs (Phase I and II) included alopecia, hiccups or sneezing after dosing, aspiration of the test material after dosing, red mucoid tissue in pan, and blood in perineal region.

Body Weight: Compound-related decreases in body weight gain were observed at 180, 538, and 1,000 mg/kg/day in a dose-dependent manner. Summaries of maternal body weight gain data for selected intervals are presented in Tables 2 and 3.

In Phase I, body weight never differed significantly from control (data not shown). However, body weight gain decreased significantly on GDs 7-10 at 180, 538, and 1,000 mg/kg/day and on GDs 7-20 at 1,000 mg/kg/day (Table 2). It increased significantly on GDs 20-28 at 538 and 1,000 mg/kg/day resulting in equal weight gains for all groups when calculated for GDs 0-28.

In Phase II, body weight decreased significantly on GD 10, 16, and 20 at 1,000 mg/kg/day (data not shown). Body weight gain decreased significantly on GDs 7-10 at 180, 538, and 1,000 mg/kg/day and on GDs 7-20 at 538 and 1,000 mg/kg/day (Table 3). It increased significantly on GDs 20-28 at 538 and 1,000 mg/kg/day resulting in equal weight gains for all groups when calculated for GDs 0-28.

Food Consumption: Compound-related effects in food consumption were observed at 538 and 1,000 mg/kg/day in a dose-dependent manner. A summary of food consumption data (only reported for Phase II) is presented in Table 4. The data were not statistically analyzed but only presented as descriptive statistics[1]. During the dosing period (GDs 7-20), food consumption decreased by 8%-28% at 538 mg/kg/day and by 40%-76% at 1,000 mg/kg/day when compared to controls. In addition, during the first

[1](i.e., means ± S.D.)

three days of dosing a 10% decrease was observed at 180 mg/kg/day, which was also believed to be compound related. During the postdosing period, compensatory increases were observed at 538 mg/kg/day during GDs 22-28 (8%-19%) and at 1,000 mg/kg/day during GDs 23-27 (10%-20%).

Gross Pathology Observations: No compound-related gross pathology was observed in either Phase I or Phase II. Common findings included pale liver, distended gallbladder, watery cecal contents, and pale kidneys.

Cesarean Section Observations: No compound-related effects were observed in any parameter. Summaries of cesarean section data are presented in Tables 5 and 6.

In Phase I, significant increases in resorptions overall and in litters with resorptions were observed at 180 and 1,000 mg/kg/day (Table 5). Although the number of resorptions were within the laboratory's historical range (2.8%-26; mean 9.5%), an additional study was conducted to determine whether this effect was compound related and could be reproduced. In Phase II, no increase was noted in resorptions (Table 6). Distributions of the sex ratio were significantly different from control at 180 mg/kg/day in Phase I and at 538 mg/kg/day in Phase II. These effects were considered to be incidental.

Developmental Toxicity

No compound-related anomalies were observed in either Phase I or Phase II studies. Incidences of external, visceral, and skeletal malformations are presented in Tables 7 and 8.

External Examinations: Phase I external malformations (Table 7) consisted of 1 fetus each at 0 and 538 mg/kg/day with omphalocele; no variations were noted (data not shown). In Phase II (Table 8), 1 fetus each at 538 and 1,000 mg/kg/day experienced omphalocele. In addition, 1 fetus in the control group had a cleft palate. Phase II variations were limited to corneal opacity which occurred with similar incidences in all groups (data not shown).

Visceral Examinations: Phase I visceral malformations (Table 7) consisted of missing caudal lung lobe in 4 (4 litters), 12 (8 litters), 3 (3 litters), and 8 (6 litters) fetuses at 0, 180, 538, and 1,000 mg/kg/day, respectively and retroesophageal right subclavian artery in 1, 1, and 2 (2 litter) fetus(es) at 180, 538, and 1,000 mg/kg/day, respectively. Additional malformations, occurring as single events, consisted of missing gallbladder and ectopic kidneys in the control group; enlarged right atrium at 538 mg/kg/day; and persistent right 4th aortic arch and missing cartilagenous rings in the trachea at 1,000 mg/kg/day. Variations, observed with similar incidences in all groups, included retrocaval ureter and pale spleen (data not shown).

Phase II visceral malformations (Table 8) consisted of missing caudal lung lobe in 2 (2 litters), 1, 1, 5 (2 litters) and 7 (4 litters) fetuses at 0, 54, 180, 538, and 1,000 mg/kg/day, respectively and retroesophageal right subclavian artery in 2 (2 litter) and 1 fetus(es) at 180 and 538 mg/kg/day, respectively. Additional malformations, occurring as single events, consisted of retroesophageal aortic arch, ectopic kidneys

and persistent truncus arteriosis at 54 mg/kg/day; diaphragmatic hernia and hypoplastic lungs at [illegible] mg/kg/day; and hemorrhagic liver at 1,000 mg/kg/day. Variations, observed with similar incidences in all groups, were limited to retrocaval ureters (data not shown).

Skeletal Examinations: Phase I skeletal malformations (Table 7) were observed in 1 fetus (hemivertebrae) at 1,000 mg/kg/day. Phase I variations, occurring with similar incidences in all groups, included delayed ossification of the hyoid and crooked hyoid; delayed ossification of the sternebrae; and lumbar and sacral spurs (data not shown).

Phase II skeletal malformations (Table 8) were observed in 1 fetus at 54 mg/kg/day (thoracic hemivertebrae and extra lumbar rib); 1 fetus at 538 mg/kg/day (thoracic hemivertebrae); and 2 fetuses (2 litters) at 1,000 mg/kg/day (one had cervical and thoracic hemivertebrae and fused caudal vertebrae, the other had fused ribs). Phase II variations, occurring with similar incidences in all groups included delayed ossification of the hyoid and crooked hyoid; extra site of ossification in the atlas; cervical and lumbar spurs; and delayed and irregular patterns of ossification in the sternebrae (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. All criteria were satisfied for both Phase I and Phase II studies.

Test Material Analyses

Analyses for concentration and homogeneity of the test material in the vehicle exceeded the commonly accepted ±10% variation on several occasions in both Phase I and Phase II studies. This may indicate that the solutions were prepared in an inconsistent manner or that instruments were not calibrated properly. Since the Phase I study was repeated with similar results, the reviewers believe that these variations did not impact negatively upon the outcome of the studies.

Maternal Toxicity

Compound-related maternal toxicity was observed at 180, 538, and 1,000 mg/kg/day. It was manifested as an increased rate of abortions (1,000 mg/kg/day); decreased body weight gain (significant) and food consumption (≥180 mg/kg/day) during the dosing period (especially during the three first days of dosing); and increased rates of clinical signs (538 and 1,000 mg/kg/day). The increased abortion rate, more pronounced in Phase II (12%) and outside the historical range (0%-8%), was considered to be a compound-related effect. Based on these results, the maternal NOEL and LOEL were 54 and 180 mg/kg/day, respectively.

Developmental Toxicity

Deaths/Resorptions: No compound-related effects were observed. The increased rates of resorptions in Phase I at 180 and 1,000 mg/kg/day were within the historical control range; were not dose-dependent; and were not reproduced in the Phase II study. In addition, the rate in the control group was below the historical control range. Therefore, these increases were considered to be spontaneous in nature.

Altered Growth: No compound-related effects were observed.

Developmental Anomalies: No compound-related effects were observed.

Based on these results, the NOEL for developmental toxicity was 1,000 mg/kg/day (limit dose).

Design/Reporting Deficiencies:

Food consumption data was recorded for the Phase II study only. However, the data provided limited information since they were not statistically analyzed.

Analytical chemistry data on dosing solutions from Phase I demonstrated variability that exceeded ±10%. Therefore, in Phase II, great care should have been taken in preparations and analyses of dosing solutions to avoid this problem. On the contrary, the variability was greater in Phase II than in Phase I indicating that no extra precautions were evoked.

An additional minor reporting deficiency included a lack of information on temperature and humidity in the animal quarters.

E. CORE CLASSIFICATION: Core Guideline Data.

Maternal NOEL = 54 mg/kg/day
Maternal LOEL = 180 mg/kg/day (decreased body weight gain and food consumption; increased clinical signs and abortions)

Developmental Toxicity NOEL = 1,000 mg/kg/day
Developmental Toxicity LOEL = Not determined

F. RISK ASSESSMENT: Not applicable

TABLE 1. Summary of Phase II (I) Clinical Signs[a]

Parameter	Dose Level (mg/kg/day) 0	54	180	538	1,000
No. animals examined	18 (18)[b]	18 (18)	13 (18)	18 (18)	18 (18)
Decreased feces	2 (1)	4	5 (1)	13 (6)	18 (5)
Soft feces	1	3	1	2	6
Feces mucoid	0	0	0	0	3
Hiccuped after dosing	0	0	1	0	0
Aspirated test material after dosing	0	0	0	1	0
Blood in pan	0	0	0	0	2
Red mucoid tissue in pan	1	0	0	0	1
Perineal staining	0	0	0	0	7
Alopecia	0	0	0	0	1

[a]Data were extracted from Study No. K-049877-015, Tables 4 and 13.

[b]Numbers within parentheses represent observations from the Phase I study.

Table 2. Mean Body Weight Gain (g ± S.D.) - Phase I[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-7)	Early Dosing Period (GD 7-10)	Entire Dosing Period (GD 7-20)	Post-Dosing Period (GD 20-28)	Entire Gestation Period (GD 0-28)
0	227 ± 110	63 ± 52	204 ± 137	71 ± 161	502 ± 197
180	258 ± 120	-24 ± 110*	145 ± 120	162 ± 65	565 ± 176
538	237 ± 107	-90 ± 128*	135 ± 112	186 ± 88*	558 ± 188
1,000	264 ± 114	-211 ± 83*	-80 ± 253*	266 ± 132*	489 ± 242

[a]Data were extracted from Study No. K-040877-015, Table 6.

*Significantly different from control ($p \leq 0.05$)

Table 3. Mean Body Weight Gain (g ± S.D.) - Phase II[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-7)	Early Dosing Period (GD 7-10)	Entire Dosing Period (GD 7-20)	Post-Dosing Period (GD 20-28)	Entire Gestation Period (GD 0-28)
0	239 ± 213	41 ± 30	196 ± 171	46 ± 125	482 ± 347
54	270 ± 105	19 ± 65	172 ± 118	126 ± 130	566 ± 172
180	281 ± 161	-5 ± 35*	201 ± 125	96 ± 125	577 ± 299
538	251 ± 207	-85 ± 111*	56 ± 116*	192 ± 80*	498 ± 242
1,000	281 ± 160	-292 ± 110*	-270 ± 341*	236 ± 176*	278 ± 376

[a]Data were extracted from Study No. K-040877-015, Table 16.

*Significantly different from control ($p \leq 0.05$)

Table 4. Food Consumption (g/animal/day ± S.D.)[a,b,c] - Phase II

Gestation Days	Dose Levels (mg/kg/day)				
	0	54	180	538	1,000
7-8	192 ± 28	190 ± 37	174 ± 45	137 ± 66	46 ± 39
9-10	194 ± 30	176 ± 46	175 ± 30	149 ± 56	80 ± 64
11-12	179 ± 28	164 ± 32	166 ± 42	165 ± 34	107 ± 69
13-14	171 ± 39	154 ± 50	165 ± 36	140 ± 35	97 ± 79
15-16	170 ± 50	150 ± 73	165 ± 59	129 ± 59	87 ± 81
17-18	161 ± 78	166 ± 63	172 ± 43	139 ± 55	87 ± 72
19-20	169 ± 69	176 ± 49	177 ± 32	130 ± 52	89 ± 88

[a]Data were extracted from Study No. K-049877-015, Table 14.

[b]Data not statistically analyzed

[c]Values for several dams were excluded.

TABLE 5. Cesarean Section Observations - Phase I[a,b]

Parameter	Dose Level (mg/kg/day) 0	180	538	1,000
No. animals mated	18	18	18	18
No. animals pregnant	18	18	16	16
Pregnancy rate (%)	100	100	89	89
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	0	0	2	2
No. aborted	0	0	0	1
Gravid uterine weight (g)	365	391	400	423
Does with live litters	18	18	16	15
Total corpora lutea[c]	193	208	189	176
Corpora lutea/doe	10.7 ± 2.4[d]	11.6 ± 2.6	11.8 ± 2.4	11.7 ± 2.3
Total implantations[c]	110	125	114	120
Implantations/doe	6.1 ± 2.6	6.9 ± 2.6	7.1 ± 2.7	8.0 ± 1.9
Total live fetuses[c]	108	113	102	103
Live fetuses/doe	6.0 ± 2.5	6.3 ± 3.3	6.4 ± 2.7	6.9 ± 2.5
Total resorptions	2	12**	12	17**
Litters with resorptions	2	8**	5	9**
Resorptions/doe	0.1 ± 0.3	0.7 ± 0.9	0.8 ± 1.5	1.1 ± 1.3
Total dead fetuses	0	0	0	0
Fetal weight/litter (g)	39.7 ± 4.8	40.4 ± 4.1	40.2 ± 4.6	38.0 ± 2.6
Preimplantation loss (%)	39	40	38	31
Postimplantation loss (%)[c]	2	10	11	14
Sex ratio (% male)	57	62[s]	46	41

[a]Data were extracted from Study No. K-049877-015, Table 8 and Table A-5.

[b]Nonpregnant animals were excluded from the analysis.

[c]Calculated by the reviewers

[d]Mean ± S.D.

[s]Distribution significantly different from binomial distribution

**Significantly different from control ($p \leq 0.01$)

TABLE 6. Cesarean Section Observations - Phase II[a,b]

Parameter	Dose Level (mg/kg/day) 0	54	180	538	1,000
No. animals mated	18	18	18	18	18
No. animals pregnant	15	13	15	18	17
Pregnancy rate (%)	83	72	83	100	94
Maternal wastage					
No. died/nonpregnant	0	0	0	0	0
No. died/pregnant	0	0	0	0	0
No. nonpregnant	3	5	3	0	1
No. aborted	0	0	0	0	2
Gravid uterine weight (g)	323	390	367	368	346
Does with live litters	12	12	13	18	12
Total corpora lutea[c]	109 (12)[d]	118 (12)	127 (13)	188 (18)	128 (12)
Corpora lutea/doe	9.1 ± 2.5[e]	9.8 ± 2.5	9.8 ± 2.8	10.4 ± 2.6	10.7 ± 1.6
Total implantations[c]	65 (13)	82 (13)	81 (13)	118 (18)	82 (14)
Implantations/doe	5.0 ± 3.0	6.3 ± 2.1	6.2 ± 2.0	6.6 ± 3.3	5.9 ± 3.1
Total live fetuses[c]	61 (13)	74 (13)	74 (13)	99 (18)	76 (14)
Live fetuses/doe	4.7 ± 3.1	5.7 ± 2.4	5.7 ± 2.2	5.5 ± 2.5	5.4 ± 3.3
Total resorptions	4 (13)	8 (13)	7 (13)	19 (18)	6 (14)
Litters with resoprtions	4	5	6	8	6
Resorptions/doe	0.3 ± 0.5	0.6 ± 1.0	0.5 ± 0.7	1.1 ± 1.8	0.4 ± 0.5
Total dead fetuses	0	0	0	0	0
Fetal weight/litter (g)	39.3 ± 10.6	41.1 ± 3.0	41.8 ± 4.9	41.5 ± 5.5	34.2 ± 8.4
Preimplantation loss (%)	43	29	40	38	37
Postimplantation loss (%)[c]	6	9	11	16	7
Sex ratio (% male)	46	46	39	64[f]	59

[a]Data were extracted from Study No. K-049877-015, Table 18 and Table A-11.

[b]Nonpregnant animals and animals detected as being pregnant with stain were excluded from the analysis.

[c]Calculated by the reviewers

[d]Number litters included in analysis

[e]Mean ± S.D.

[f]Distribution significantly different from binomial distribution

TABLE 7. Incidences of Fetal Malformations - Phase I[c]

Findings[b]	Dose Level (mg/kg/day) 0	180	538	1,000
No. fetuses (litters) examined	108 (18)	113 (18)	102 (16)	103 (15)
External Malformations				
Omphalocele	1	0	1	0
Total no. fetuses (litters) with any external malformation(s)	1	0	1	0
Visceral Malformations				
Retroesophageal right subclavian artery	0	1	1	2 (2)
Enlarged right atrium	0	0	1	0
Missing caudal lung lobe	4 (4)	12 (8)	3 (3)	8 (6)
Missing gallbladder	1	0	0	0
Ectopic kidneys	1	0	0	0
Persistant right 4th aortic arch	0	0	0	1
Missing cartilagenous rings in trachea	0	0	0	1
Total no. fetuses (litters) with any visceral malformation(s)	6 (6)	12 (8)	5 (5)	10 (7)
Skeletal Malformations				
Hemivertebrae	0	0	0	1
Total no. fetuses (litters) with any skeletal malformation(s)	0	0	0	1
Total no. fetuses (litters) with any malformation(s)	7 (7)	12 (8)	6 (6)	10 (7)

[a]Data were extracted from Study No. K-049877-015, Table 10 and individual animal data.

[b]More than one type of anomaly may be found in one fetus.

TABLE 8. Incidences of Fetal Malformations - Phase II[a]

Findings[b]	Dose Level (mg/kg/day) 0	54	180	538	1,000
No. fetuses (litters) examined	61 (12)	74 (12)	74 (13)	99 (18)	76 (12)[c]
External Malformations					
Omphalocele	0	0	0	1	1
Cleft palate	1	0	0	0	0
Total no. fetuses (litters) with any external malformation(s)	1	0	0	1	1
Visceral Malformations					
Missing caudal lung lobe	2 (2)	1	1	5 (2)	7 (4)
Retroesophageal right subclavian artery	0	0	2 (2)	1	0
Retroesophageal aortic arch	0	1	0	0	0
Ectopic kidney	0	1	0	0	0
Persistent truncus arteriosis	0	1	0	0	0
Diaphragmatic hernia	0	0	0	1	0
Hypoplastic lungs	0	0	0	1	0
Liver hemorrage	0	0	0	0	1
Total no. fetuses (litters) with any visceral malformation(s)	2 (2)	4 (4)	3 (3)	7 (4)	7 (4)
Skeletal Malformations					
Hemivertebrae, cervical	0	0	0	0	1
Hemivertebrae, thoracic	0	1	0	1	1
Fused caudal vertebrae	0	0	0	0	1
Extra Lumbar rib	0	1	0	0	0
Fused rib	0	0	0	0	1
Total no. fetuses (litters) with any skeletal malformation(s)	0	1	0	1	2 (2)
Total no. fetuses (litters) with any malformation(s)	3 (3)	5 (5)	3 (3)	9 (6)	9 (5)

[a]Data were extracted from Study No. K-049877-015, Table 19 and individual animal data.

[b]More than one anomaly may be found in one fetus.

[c]In the 1,000 mg/kg/day dose group only 70 fetuses (11 litters) were subjected to a skeletal examination.

ATTACHMENT I

010198

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with a * are supplemental, may not be required for every study.